

The human *patched* gene has been mapped to human chromosome band 9q22.3, and lies between the polymorphic markers D9S196 and D9S287 (a detailed map of human genome markers may be found in Dib et al. (1996) Nature 280:152 —<http://www.genethon.fr>).

In the claims:

For the convenience of the Examiner, all claims whether or not amended are presented below.

Please cancel, without prejudice, claim 69.

61. **(Four Times Amended)** A method for identifying an agent which decreases hedgehog signal transduction for ameliorating an affect of loss of function of a patched gene in a cell, comprising contacting one or more test agents with a cell that expresses a wild-type patched protein and identifying one or more test agents that decrease the level of hedgehog signal transduction relative to the absence of test agent, wherein an agent that decreases hedgehog signal transduction is useful for ameliorating an affect in a cell characterized by loss of function of a patched gene.

62. **(Four Times Amended)** A method for identifying an agent which decreases hedgehog signal transduction for ameliorating an affect of loss of function of a patched gene in a cell, comprising contacting one or more test agents with a cell characterized by loss of function of a patched gene and identifying one or more test agents that decrease the level of hedgehog signal transduction relative to the absence of test agent, wherein an agent that decreases hedgehog signal transduction is useful for ameliorating an affect in a cell characterized by loss of function of a patched gene.

63. **(Four Times Amended)** A method for identifying an agent which decreases hedgehog signal transduction in a cell characterized by loss of function of a patched gene, comprising:

- a. comparing the amount of expression of a reporter gene in a recombinant mammalian cell in the presence of a test agent with the amount of expression in the absence of the agent; and

b. identifying test agents that decrease the amount of expression of the reporter gene in the recombinant cell in the presence of the agent compared to the amount of expression in the absence of the agent, wherein:

the recombinant cell contains a reporter gene construct and said recombinant cell does not express a functional wild-type patched protein; and

the reporter gene construct contains:

- (i) a transcriptional control element that is stimulated by hedgehog signal transduction; and
- (ii) a reporter gene that encodes a detectable product and that is in operative association with the transcriptional control element;

wherein a test agent identified in step (b) decreases hedgehog signal transduction in a cell characterized by loss of function of a patched gene.

64. **(Reiterated)** The method of claim 63, wherein the amount of transcription is determined by measuring the amount of mRNA that is transcribed from said reporter gene.

65. **(Amended)** The method of claim 63, wherein the amount of transcription is measured by measuring the amount of a reporter gene protein that is produced.

66. **(Reiterated)** The method of claim 63, further comprising, prior to comparing the difference in the amount of transcription of the reporter gene, contacting the recombinant cell with a hedgehog agonist in an amount sufficient to change the level of transcription of said reporter gene.

67. **(Reiterated)** The method of claim 63, wherein the reporter gene is at least one of a gene encoding chloramphenicol acetyltransferase, a gene encoding firefly luciferase, a gene encoding bacterial luciferase, or a gene encoding alkaline phosphatase.

68. **(Reiterated)** The method of claim 63, wherein the transcriptional control region includes at least one regulatory element selected from transcriptional regulatory elements of a patched gene, transcriptional regulatory elements of a gli gene, or transcriptional regulatory elements of a PTHrP gene.

U.E.
70. **(Reiterated)** The method of claim 63, wherein expression of the reporter gene occurs upon hedgehog stimulation, and compounds are selected by ability to inhibit the expression of the reporter gene.

71. **(Reiterated)** The method of claim 63, wherein the cell characterized by a loss of function of a patched gene is a basal cell carcinoma cell.

F4
72. **(Twice Amended)** A method for preparing an agent for inhibiting growth of cells characterized by loss of function of a patched gene, comprising:

- a. contacting one or more test agents with a cell that expresses a wild-type patched protein and identifying test agents that decrease the level of hedgehog signal transduction relative to the absence of test agent;
- b. contacting test agents identified in step (a) with a cell having a loss of function of a patched gene and selecting those test agents that inhibit growth of cells having a loss of function of a patched gene; and
- c. preparing a formulation including a test agent that inhibits the growth of cells selected in step (b) and a pharmaceutically acceptable diluent.

F5
73. **(Amended)** The method of claim 72, wherein the cell having a loss of function of a patched gene is a basal cell carcinoma cell.

74. **(Reiterated)** The method of claim 61 or 62, further comprising preparing a formulation including an agent which decreases hedgehog signal transduction and a pharmaceutically acceptable excipient.

U.E.
75. **(Reiterated)** The method of claim 74, further comprising administering the formulation to a patient.

76. **(Reiterated)** The method of claim 63, further comprising preparing a formulation including an agent which decreases hedgehog signal transduction and a pharmaceutically acceptable excipient.

N.E.
77. (Reiterated) The method of claim 76, further comprising administering the formulation to a patient.

The amended claims are re-stated below to reflect changes from the last filing.

61. (Four Times Amended) A method for identifying an agent which decreases hedgehog signal transduction for ameliorating an affect of loss of function of a patched gene in a cell, comprising contacting one or more test agents with a cell that expresses a wild-type patched protein and identifying one or more test agents that decrease the level of hedgehog signal transduction relative to the absence of test agent, wherein an agent that decreases hedgehog signal transduction is useful for ameliorating an affect in a cell characterized by loss of function of a patched gene.

62. (Four Times Amended) A method for identifying an agent which decreases hedgehog signal transduction for ameliorating an affect of loss of function of a patched gene in a cell, comprising contacting one or more test agents with a cell ~~having a~~ characterized by loss of function of a patched gene ~~loss-of-function phenotype~~ and identifying ~~these~~ one or more test agents that decrease the level of hedgehog signal transduction relative to the absence of test agent, wherein an agent that decreases hedgehog signal transduction is useful for ameliorating an affect in a cell characterized by loss of function of a patched gene.

63. (Four Times Amended) A method for identifying an agent which decreases hedgehog signal transduction in a cell characterized by loss of function of a patched gene, comprising:

- comparing the amount of expression of a reporter gene in a ~~first~~ recombinant mammalian cell in the presence of a test agent with the amount of expression in the absence of the agent, ~~or with the amount of reporter gene expression in a second recombinant cell;~~ and
- identifying test agents that decrease the amount of expression of the reporter gene in the ~~first~~ recombinant cell in the presence of the agent compared to the amount of expression in the

absence of the agent, ~~or compared to the amount of reporter gene transcription or product in the second recombinant cell~~, wherein:

the ~~first~~ recombinant cell contains a reporter gene construct and ~~expresses patched~~;
~~the second recombinant cell is identical to the first recombinant cell, except that it said~~
recombinant cell does not express a functional wild-type patched protein; and

the reporter gene construct contains:

- (i) a transcriptional control element that is stimulated by hedgehog signal transduction; and
- (ii) a reporter gene that encodes a detectable product and that is in operative association with the transcriptional control element;

wherein a test agent identified in step (b) decreases hedgehog signal transduction in a cell characterized by loss of function of a patched gene.

65. **(Amended)** The method of claim 63, wherein the amount of transcription is measured by measuring the amount of a reporter gene protein that is produced.

72. **(Twice Amended)** A method for preparing an agent for inhibiting growth of cells characterized by loss of function of a patched gene, comprising:

- a. contacting one or more test agents with a cell that expresses a wild-type patched protein and identifying test agents that decrease the level of hedgehog signal transduction relative to the absence of test agent;
- b. contacting test agents identified in step (a) with a cell having a ~~patched~~ loss[-] of [-] function of a patched gene phenotype and selecting those test agents that ~~reverse at least in part the patched~~ inhibit growth of cells having a loss [-] of [-] function of a patched gene phenotype; and
- c. preparing a formulation including a test agent that inhibits the growth of cells selected in step (b) and a pharmaceutically acceptable diluent.

73. **(Amended)** The method of claim 72, wherein the cell having a ~~patched~~ loss [-] of [-] function of a patched gene phenotype is a basal cell carcinoma cell.